

## A Stem Gall on *Muehlenbeckia australis* (Forst. f.) Caused by the Moth *Morova subfasciata* Walk.

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THE RAMPANT, much-branched liane *Muehlenbeckia australis* is a familiar sight in many New Zealand forests, reaching into the tops of trees 30 feet high with woody ropelike stems, or heaped in a tangled mass where no support is provided by other plants.

Among the branches of *Muehlenbeckia australis* are sometimes found persistent woody hypertrophies which arise as spindle-shaped or nut-shaped galls on one-year-old stems. The diameter of the young galls may be about five times that of the stems on which they arise (Fig. 1).

During the first year of growth, galls of *M. australis* are found to contain either the dark pupa of a moth, or the pale larva (Fig. 2) which actively feeds on the tissues of the pith. An exit passage for the later escape of the adult moth runs from the centre of the gall towards the surface, but stops short, separated from the outside wall by a thin membrane.

Galls from which the small tawny moths (Fig. 3) have emerged can be recognized by the open escape hole.

In a discussion on gall insects, Brues (1946) made a comparison of plant galls with animal tumours, stating that "the gall continues to develop only under the sustained action of the stimulating agent, while the growth of the animal neoplasm is not thus limited." Brues did not refer to the well known exception of crown gall (Mani, 1964), probably because of its relation to bacteria and not to insects. Brues' claim may be valid in a general way since few cases of the continued growth of galls in the absence of the causal agent have been reported. Undoubtedly the moth-induced gall of *M. australis* is one of the few known exceptions.

One aim of this study was to examine the histological make-up of the galls for any evi-

dence of cellular transformation which might be associated with differences in growth between the gall and the normal stem. A second objective was to test extracts of the moth larvae for growth-stimulating capacity on stems of seedlings of *M. australis*. Finally, it was hoped to induce galls under laboratory conditions with young living larvae, preferably newly hatched from eggs.

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### METHODS AND MATERIALS

Four dozen seedlings about 6 inches in length were dug up in the forest and grown separately in 4-inch plastic pots in a glasshouse in a mixture of gravel, sand, and garden loam. Growth over 18 months was active and healthy.

Five active larvae were removed from their galls and immersed entirely in 1 ml pure 100% acetone. The larvae were crushed thoroughly in the acetone in a glass vial with a glass rod and were left in the stoppered vial for a week. The acetone was evaporated with the temperature at 25°C. To the dry residue of the ground-up larvae in the original vial was added 2 ml anhydrous lanolin, which was mixed thoroughly with the larval material (using a glass rod) to give even dispersal.

To each of 12 *Muehlenbeckia* seedlings a small globule of the paste mixture was added in the axil of a young leaf near the stem tip.

With 12 other *Muehlenbeckia* seedlings, the tip of a shoot was removed in front of a node and a small blob of the paste mixture was placed on the cut surface on each plant.

On 12 other seedlings small blobs of paste mixture were applied to the abaxial surface in the centre of young leaves.

The remaining 12 seedlings were treated on cut shoot surfaces with plain lanolin lacking larval extract.

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Cuttings of stems bearing galls with larvae were obtained with about 20% success by placing the specimens in jars of clean tap water which was changed two or three times a week. Roots appeared within 14 days at room temperature.

Moths were raised under large bell jars in which gall-bearing twigs were enclosed, with the basal end of the twig in wet sand.

On the first occasion in March a female and a male moth hatched within 24 hours of each other. They were placed overnight in a separate bell jar containing fresh *Muehlenbeckia* shoots set in moist sand. In the morning some 30 orange-coloured eggs were found, laid in short rows, some on the glass walls of the bell jar and some on the *Muehlenbeckia* stems. Both moths were dead by the following day. Only one other moth hatched from the galls on this occasion, and that was a solitary female which emerged a few days later.

On a second occasion, in December, a male and a female moth emerged about the same time under bell jars in the laboratory. They were placed overnight in a covered beaker with a fresh leaf of *Muehlenbeckia*. Again eggs were laid, all of them on the walls of the beaker, but they were almost white, lacking the colour of those laid in March.

No eggs hatched, either of the white or the orange batch.

An attempt to induce galls artificially was made by removing three small active larvae from galls and placing them on separate plump softwood cuttings of young *M. australis* shoots. The shoots were kept in large covered glass jars with the cut ends immersed in small flasks of fresh tap water. Overnight the larvae burrowed into the stems, entering near the axil of the first prominent leaf. After 24 hours the entry holes eaten by the larvae had been closed by a smooth dark membrane. Within a week the shoots with the larvae inside had blackened and were beginning to die. Control shoots lacking the larvae also died a few days later.

Material of galls and normal stems for histological examination was fixed in Formo-acetic-alcohol, embedded in paraffin, sectioned serially at 10 $\mu$  and stained in safranin and fast green (Johansen, 1940).

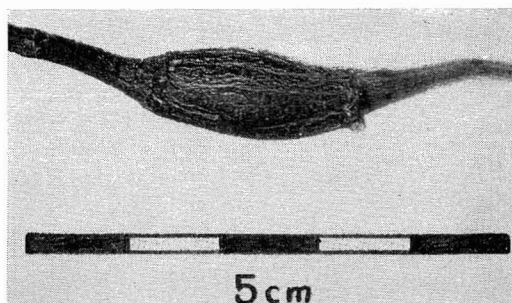


FIG. 1. A gall still occupied by the larval moth.

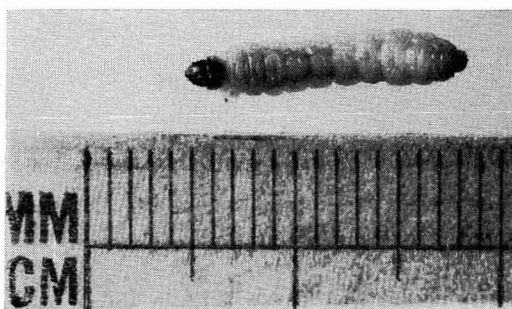


FIG. 2. Larva of *Morova subfasciata*.

#### OBSERVATIONS

Male adult moths were smaller, darker, and more active than females (Fig. 3). Neither survived more than a day after the eggs were laid.

The dates of emergence of the moths from galls were in agreement with Hudson's report (1928) that the perfect insect is to be found from December to March. Entomological details of the dark brown insect pupa and the pale larva (Fig. 2) are included in Hudson's descriptions and are not necessary here.

Since the eggs laid in the laboratory did not hatch, it was decided to use larvae from existing galls to attempt to induce new galls artificially. The results of allowing larvae removed from galls to penetrate cuttings of *Muehlenbeckia* shoots were inconclusive because the cuttings did not live long enough for galls to form. (The control cuttings also survived only a short time.)

However, it was seen that the larvae retained the ability to eat instinctively into the pith and to seal the entry hole. (When similar experiments were carried out by the author with larvae

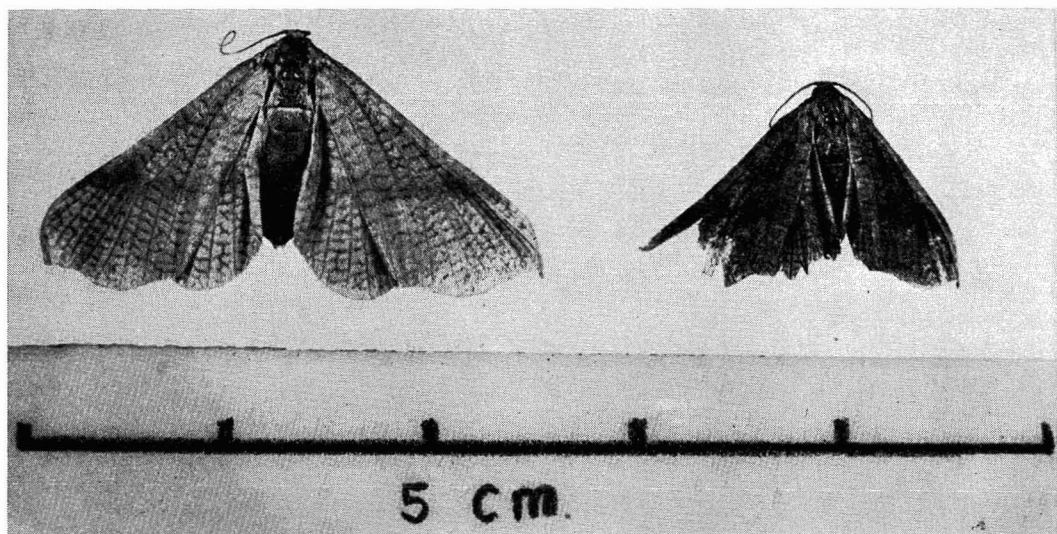


FIG. 3. Adult moths, female on the *left*.

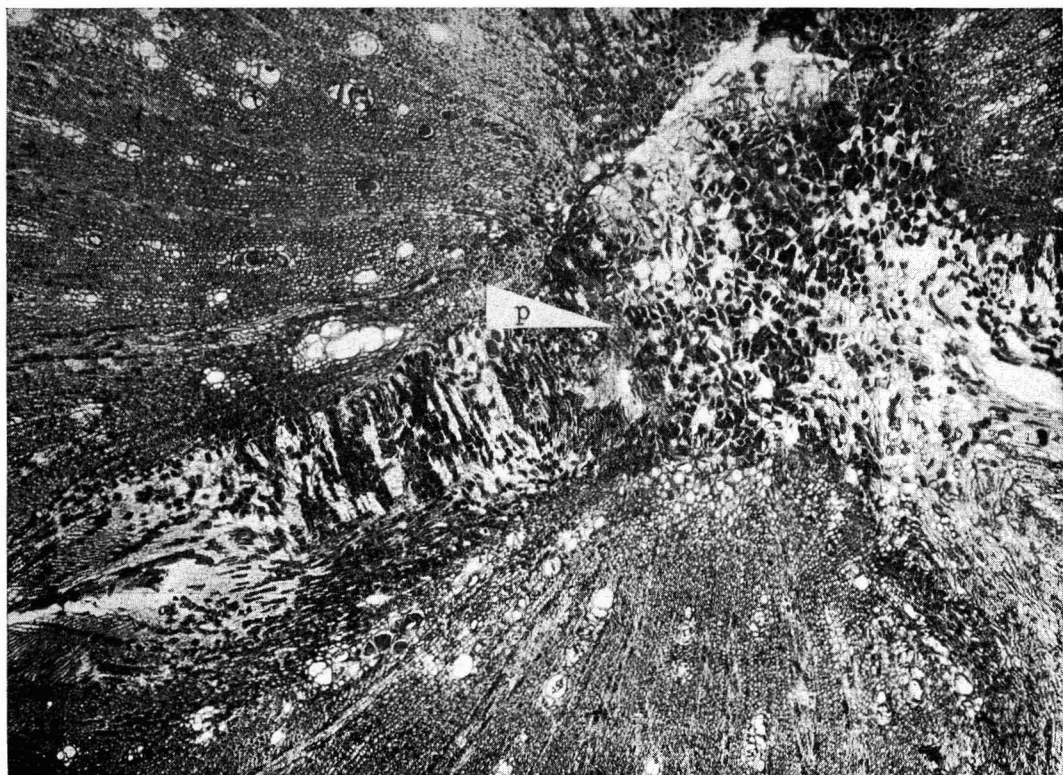


FIG. 4. Transverse section of gall showing invasive pith cells (*p*, pith cells).

of a species of gall midge, they failed to re-enter new shoots of the host plant.) With the larvae of *Morova subfasciata*, however, further work with intact seedlings, rather than cuttings, of *M. australis* might give promising results under laboratory or glasshouse conditions.

Such experiments depending on the feeding action of the larvae may be more fruitful than attempts to induce galls with larval extracts. In the experiments in which crude acetone extracts of larvae were applied to seedlings of *M. australis*, there was no sign of growth stimulation in the treated plants after 18 months, at which time the 48 seedlings were discarded.

Sustenance for the larva is provided by the continued growth of the pith cells of the gall. When living galls are cut open and kept moist in a petri dish, after removal of the larva, white callus-like proliferations of the pith become apparent to the naked eye after a week at room temperature. (If the larva is left inside a gall from which only a "window" of tissue has been removed, the larva forms a smooth dark membrane resealing the cavity in a few hours.) No callus-like proliferations are obtained from living galls from which the moth has departed; and it appears that the larva eventually consumes the entire pith tissue within the length of the gall.

A comparison of the anatomy of the gall with that of normal stems of *M. australis* shows some interesting differences which seem to account for the process of gall enlargement.

Serial cross sections through young galls and the adjacent portions of the stem show that the presence of the larva is associated with excessive growth of vascular rays and pith. The pith and vascular rays enlarge and actively invade the woody cylinder (Fig. 4), pushing outward to the cortex. At the same time, or shortly afterwards, the cambium itself becomes overactive, giving rise to further ray tissue of irregular starch-packed parenchyma. The end result of this excessive growth of pith, rays, and cambium is that the vascular cylinder is cleft into several cable-like strands (Fig. 5) which traverse the body of the gall like a cage around the larva.

In older normal stems of *M. australis* the

vascular cylinder resembles a scalloped column. Presumably this is the result of the cambium forming larger proportions of xylem than of phloem at the semicircular xylem lobes, and a corresponding excess of phloem in the V-shaped grooves between the lobes (Eames and McDaniels, 1925). This atypical growth of the cambium is associated with the laying down of conspicuous rays which are continuous in spoke-like fashion (in cross section) from the pith to the grooves between the xylem lobes.

Nevertheless, the normal stem of *M. australis* is a compact unity and not a composite of separated strands like the body of the gall or the typical stems of other lianes. It is well known that in several lianes anomalous activity of the cambium may give rise to a separation of the vascular system into ropelike strands (Eames and McDaniels, 1925).

Thus, the morphogenetic influence of the moth has been to initiate abnormally high growth rates and new growth patterns in the pith, rays, and cambium; and to produce the counterpart of advanced liane-type anatomy in a plant which by itself has not evolved far in this direction.

#### DISCUSSION

The tendency of the cambium of the normal stem of *M. australis* to produce conspicuous vascular rays and varying proportions of xylem and phloem at different sites could be taken as the suggestion of an evolutionary trend toward the type of stem structure seen in lianes with a vascular system composed of separated strands. Without this slight tendency being already inherent in *M. australis*, it is doubtful whether the larva of the gall moth would elicit such a dramatic anatomical transformation.

The persistence of the galls as an overgrowth after the departure of the insect may reflect merely the continued growth of tissues after a preliminary boost and reorientation, without indicating a truly tumorous condition, and may not be entirely contradictory to the concept of tumour growth implied in the statement of Brues (1946) quoted earlier. The initial invasiveness of pith and ray cells may reflect the stimulatory effect of wound hormones resulting



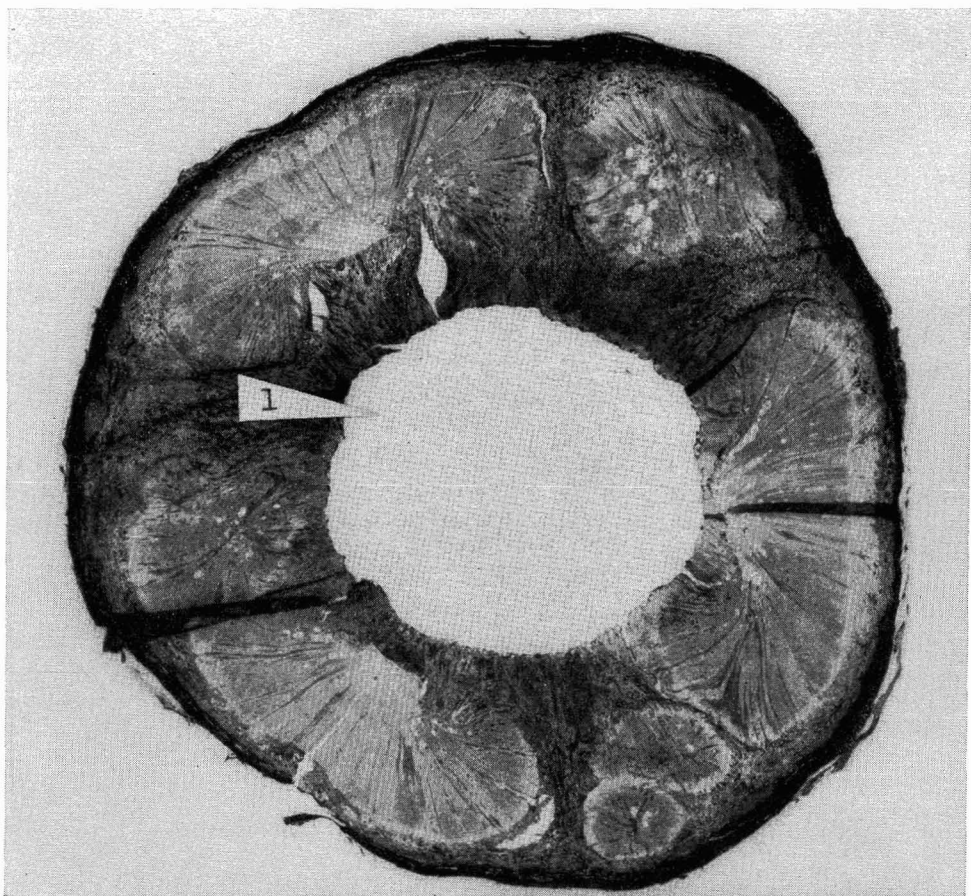


FIG. 5. Transverse section through gall showing division of vascular system into separate parts (1, larval cavity).

from the feeding action of the larva (Bloch, 1952) and may not represent a permanent cellular transformation such as appears to be the case in crown gall and in animal cancers. Electron microscope studies might prove illuminating in answering such questions, and should at least reveal the presence of any viruses or bacteria which might be associated with the larvae and which have not at present been detected.

Whatever adverse effects the larva and gall may have on *M. australis*, there is no obvious sign that translocation or transpiration is seriously impeded. This is probably because the ropelike divisions of the stele which run separately through the gall are united at either end and do not disrupt the continuity of the climbing stems which may extend to great heights.

#### SUMMARY

Stem galls on the liane *Muehlenbeckia australis* (Forst.f.) are caused by the larval form of the moth *Morova subfasciata* Walk., which feeds on proliferating tissue of the pith.

After the departure of the insect, the gall continues to grow without blocking translocation or transpiration in the stem.

Gall formation is brought about by excessive growth of the pith, vascular rays, and cambium, which separate the vascular cylinder into several ropelike portions which remain united at either end of the gall.

Applications of crude acetone extracts of young larvae failed to produce galls on seedlings of *M. australis* grown in a glasshouse.

Larvae removed from galls are capable of re-entering new stems and re-establishing themselves in the pith after sealing off the entry hole.

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